P22042.A01

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Atsushi MIYAWAKI et al.

Group Art Unit : Not Known

Appl. No. : Not Yet Assigned

Examiner : Not Known

Filed : Concurrently Herewith

For : A FLUORESCENT PROTEIN

PRELIMINARY AMENDMENT

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Prior to calculation of filing fees and an examination of the above-identified patent application, entry of the following amendment is respectfully requested.

IN THE CLAIMS

Please amend claim 21 as follows (a marked-up copy of the changes is included in the Appendix attached to the present amendment):

21. (Amended) A kit for measuring calcium ions which comprises at least one or more selected from a fluorescent protein having the following amino acid sequences (1) to (3) in order in the direction from the N-terminus to the C-terminus, wherein a fused fluorescent protein obtained by fusion of the fluorescent protein with a calcium binding protein and its target peptide can emit fluorescence which is dependent on Ca^{2+} ion level;

(1) an amino acid sequence from the n^{th} amino acid from the N-terminus to the C-terminus of a fluorescent protein selected from the group consisting of a green fluorescent

protein or its mutant, a yellow fluorescent protein or its mutant, a cyan fluorescent protein or its mutant, a red fluorescent protein or its mutant, and a blue fluorescent protein or its mutant, provided that n represents an integer of 140 to 150;

(2) a linker sequence of a sequence of 2 to 20 amino acids; and

(3) an amino acid sequence from the 1st amino acid to the (n-1)th amino acid from the N-terminus of the fluorescent protein described in (1) above;

a fused fluorescent protein having the following amino acid sequences (1) to (5) in order in the direction from the N-terminus to the C-terminus, which can emit fluorescence that is dependent on Ca^{2+} ion level.

(1) an amino acid sequence of a target peptide of a calcium-binding protein;

(2) an amino acid sequence from the nth amino acid from the N-terminus to the C-terminus of a fluorescent protein selected from the group consisting of a green fluorescent protein or its mutant, a yellow fluorescent protein or its mutant, a cyan fluorescent protein or its mutant, a red fluorescent protein or its mutant, and a blue fluorescent protein or its mutant, provided that n represents an integer of 140 to 150;

(3) a linker sequence of a sequence of 2 to 20 amino acids;

(4) an amino acid sequence from the 1st amino acid to the (n-1)th amino acid from the N-terminus of the fluorescent protein described in (2) above; and

(5) the amino acid sequence of a calcium-binding protein;

[the] a calcium ion indicator [of claims 12,] comprising the fused fluorescent protein;

a DNA encoding the fluorescent protein;

a recombinant vector having the DNA; or

a transformant having the DNA or the recombinant vector.

REMARKS

The Examiner is respectfully requested to enter the foregoing amendment to remove multiple dependent claims prior to examination of the above-identified patent application.

The amendments to the claims made in this amendment have not been made to overcome the prior art, and thus, should be considered to have been made for a purpose unrelated to patentability, and no estoppel should be deemed to attach thereto.

Should there be any questions, the Examiner is invited to contact the undersigned at the below-listed telephone number.

Respectfully submitted,
Atsushi MIYAWAKI et al.

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March 4, 2002

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APPENDIX
MARKED-UP COPY OF CHANGES TO CLAIM 21:

21. (Amended) A kit for measuring calcium ions which comprises at least one or more selected from [the] a fluorescent protein [of claim 1,] having the following amino acid sequences (1) to (3) in order in the direction from the N-terminus to the C-terminus, wherein a fused fluorescent protein obtained by fusion of the fluorescent protein with a calcium binding protein and its target peptide can emit fluorescence which is dependent on Ca^{2+} ion level;

(1) an amino acid sequence from the n^{th} amino acid from the N-terminus to the C-terminus of a fluorescent protein selected from the group consisting of a green fluorescent protein or its mutant, a yellow fluorescent protein or its mutant, a cyan fluorescent protein or its mutant, a red fluorescent protein or its mutant, and a blue fluorescent protein or its mutant, provided that n represents an integer of 140 to 150;

(2) a linker sequence of a sequence of 2 to 20 amino acids; and

(3) an amino acid sequence from the 1^{st} amino acid to the $(n-1)^{th}$ amino acid from the N-terminus of the fluorescent protein described in (1) above;

[the] a fused fluorescent protein [of claim 6,] having the following amino acid sequences (1) to (5) in order in the direction from the N-terminus to the C-terminus, which can emit fluorescence that is dependent on Ca^{2+} ion level.

(1) an amino acid sequence of a target peptide of a calcium-binding protein;

(2) an amino acid sequence from the n^{th} amino acid from the N-terminus to the C-terminus of a fluorescent protein selected from the group consisting of a green fluorescent protein or its mutant, a yellow fluorescent protein or its mutant, a cyan fluorescent protein

or its mutant, a red fluorescent protein or its mutant, and a blue fluorescent protein or its mutant, provided that n represents an integer of 140 to 150;

(3) a linker sequence of a sequence of 2 to 20 amino acids;

(4) an amino acid sequence from the 1^{st} amino acid to the $(n-1)^{th}$ amino acid from the N-terminus of the fluorescent protein described in (2) above; and

(5) the amino acid sequence of a calcium-binding protein;

[the] a calcium ion indicator [of claims 12,] comprising the fused fluorescent protein;

a [the] DNA [of claim 14,] encoding the fluorescent protein;

[the] a recombinant vector [of claim 18,] having the DNA; or

[the] a transformant [of claim 19] having the DNA or the recombinant vector.